REMARKS

Amendments

Claims 29, 30, and 32 have been canceled. Claims 24, 37, and 39 have been amended. New claim 41 has been added. Upon entry of the amendment, claims 24-26, 28, 36-39, and 41 will be pending.

The foregoing amendments are made solely to expedite prosecution of the application and are not intended to limit the scope of the invention. Further, the amendments to the claims are made without prejudice to the pending or now canceled claims or to any subject matter pursued in a related application. The Applicant reserves the right to prosecute any canceled subject matter at a later time or in a later filed divisional, continuation, or continuation-in-part application.

Specification

The amendment filed on March 7, 2005 is objected to under 35 U.S.C. § 132 because it allegedly introduces new matter into the disclosure. Applicant respectfully disagrees. With respect to the amendment to page 10, paragraph 4, Applicant notes that United States Patent Application Serial No. 08/971,310 (which was referred to in the specification as originally filed) was converted to United States Provisional Patent Application Serial No. 60/084,194. Only the disclosure of United States Provisional Patent Application Serial No. 60/084,194 is incorporated by reference, as indicated by the language "the disclosure of provisional application no. 60/084,194 which is incorporated herein by reference in its entirety" in the amendment of February 28, 2005. Mere reference to another application, patent, or publication is not an incorporation of anything therein into the application containing such reference for the purpose of the disclosure required by 35 U.S.C. 112, first paragraph. *In re de Seversky*, 474 F.2d 671, 177 USPQ 144 (CCPA 1973). Therefore, the other patents and patent applications recited in the amended paragraph are not incorporated by reference, and therefore do not constitute new matter. Withdrawal of the objection is respectfully requested.

Rejections

Rejections under 35 U.S.C. § 101

The Examiner has rejected claims 24-26, 28-30, 32, and 36-39 under 35 U.S.C. § 101 because the claimed invention is allegedly not supported by either a specific or substantial asserted utility or a well-established utility. Applicant respectfully traverses the rejection.

Applicant incorporates and references arguments made in the amendment submitted March 18, 2005. These references and arguments clearly indicate that the claimed knockout mouse has a specific and substantial utility. A further article conclusively states the utility of mouse knockouts:

> After a decade of using mouse knockouts, the data on their predictive power in drug discovery is irrefutable. The top 100 selling drugs in 2001 are directed only to 29 drug targets, many with multiple agents addressing the same target. Of these 29 targets, 23 have been knocked out and in every case the knockout mouse was highly predictive as to the on-target effects and side effects of the associated drugs.

(Arthur T. Sands, Industrializing Breakthrough Discovery, Current Drug Discovery, Aug. 2002, at 21.) (emphasis added) (copy enclosed).

Applicant submits that in light of arguments of record that a person of ordinary skill in the art would immediately appreciate why the invention is useful. Applicant submits that it cannot be reasonably debated that a person of ordinary skill in the art would not immediately appreciate why the invention is useful: for determining the function of the mTMT gene, for studying disease processes in which mTMT plays a role, and for drug discovery.

Applicant respectfully reminds Examiner that a claimed invention need only satisfy one of its stated objectives to satisfy the utility and enablement requirements. Furthermore, the Applicant reminds the Examiner that "[t]he threshold of utility is not high: An invention is 'useful' under section 101 if it is capable of providing some identifiable benefit." *Juicy Whip, Inc. v. Orange Bang, Inc.*, 185 F.3d 1364, 1366, 51 U.S.P.Q.2d 1700 (Fed. Cir. 1999). To lack utility means that the invention "must be totally incapable of achieving a claimed result." *Brooktree Corp. v. Advanced Micro Devices, Inc.*, 977 F.2d 1555,1571 (Fed. Cir. 1992).

The Examiner alleges that the phenotypic differences between the mTMT knockout mice and wild-type mice are very small and do not appear to be statistically significant. Applicant has submitted a declaration signed by John Burke, attorney of record, showing that the phenotypic differences are statistically significant. Specifically, the declaration and supporting documentation show that the increased Prepulse Inhibition (PPI) with a 90dB prepulse (as depicted in Figure 5) is statistically significant with a 1-*p* value of 0.98 (9 wild-type mice tested and 11 mTMT knockout mice tested). Thus, additional experimentation is not required to establish the phenotypic differences are of sufficient magnitude to be useful as a model.

The Examiner alleges that all of the teachings in the specification regarding the utility of the claimed animal as a disease model are general in nature and would apply to any transgenic animal with an altered phenotype. Applicant respectfully disagrees. First, one skilled in the art would immediately understand that mTMT knockout mice have a well-established utility as models of diseases. It was well known at the time of filing that tryptases are prominently expressed in mast cells, and that mast cells play beneficial immunosurveillance and effector roles in the body, especially during bacterial infections. See Caughey et al., J Immunol. 2000 Jun 15;164(12):6566-75 (copy enclosed); Malavitya et al., J Immunol. 1994 Feb 15;152(4):1907-14 (copy of abstract enclosed). Therefore, the claimed mice can be used as models of disease in which mast cell function is impaired.

Second, it was also well known in the art at the time of filing that mast cell tryptases are implicated in allergic airway diseases, including asthma. See Caughey et al, *supra*. Furthermore, it was well known that tryptase inhibitors block allergic bronchoconstriction and eosinophilic inflammation in sheep; human trials of tryptase inhibitors have shown that they reduce asthmatic responses to inhaled allergen. See Caughey et al, *supra*. Hence, one skilled in the art would immediately understand that the claimed mice can be used to validate mTMT as a "druggable" gene target. Because the claimed mice have a null allele of the mTMT gene, the claimed mice allow one to predict the likely effects—and side effects—of a drug that antagonizes mTMT function. Inhibitors of other tryptases have been developed for the treatment of asthma in humans; therefore, the mTMT knockout mice can be used to predict the effects of a specific inhibitor of TMT in humans.

Third, Applicants submit again that, independently of their use as models for mTMT-mediated disease or models of mTMT antagonists, mice having a null allele in the mTMT gene

have another inherent and well-established utility: they may be used in the study of the function of the mTMT gene. The fact that this is a well-established utility is evidenced by the references quoted in the office action response filed March 18, 2005. For example, as pointed out by Doetschman, one clearly skilled in the art, (*Laboratory Animal Science* 49:137-143, 137 (1999)(copy previously supplied), the phenotypes observed in mice do correlate to gene function:

> The conclusions will be that the knockout phenotypes do, in fact, provide accurate information concerning gene function, that we should let the unexpected phenotypes lead us to the specific cell, tissue, organ culture, and whole animal experiments that are relevant to the function of the genes in question, and that the absence of phenotype indicates that we have not discovered where or how to look for a phenotype. (emphasis added).

This is a specific utility as only mice having a disruption in the mTMT gene may be used to study the function of mTMT gene.

Furthermore, the mice of claim 38 and claim 39 have a visible marker gene inserted into the mTMT coding sequence. Expression is therefore driven by the endogenous mTMT promoter. Expression of the LacZ gene indicates where the mTMT gene is expressed, and so the mice can be used to determine the expression pattern of the gene, which is a specific, substantial and credible utility. As discussed in the Office action response dated March 18, 2005, the use of transgenic in studying gene expression is clearly recognized by those skilled in the art:

> **Null-reporter alleles should be created**
> The project should generate alleles that are as uniform as possible, to allow efficient production and comparison of mouse phenotypes. The alleles should achieve a balance of utility, flexibility, throughput and cost. A null allele is an indispensable starting point for studying the function of every gene. Inserting a reporter gene (e.g., P-galactosidase or green fluorescent protein) allows a rapid assessment of which cell types normally support the expression of that gene.

(Austin et al., Nature Genetics (2004) 36(9):921-24, 922)(emphasis in original; emphasis added)(copy previously supplied).

The Examiner argues that the utility of a given mouse for studying the utility of a given gene is not a certainty. Applicant notes that there is no requirement in the law that there must be "certainty" with regard to an asserted utility. As held by the CCPA: "Nor must an applicant

provide evidence such that it establishes an asserted utility as a matter of statistical certainty." *Nelson v. Bowler*, 626 F.2d 853, 856-57, 206 USPQ 881, 883-84 (C.C.P.A. 1980).

The Examiner also argues that the relationship of the phenotype displayed by a mouse comprising a mutation in a given gene is "highly" dependent upon genetic background and therefore the utility is not substantial. The Examiner argues, citing Gerlai et al., Trends Neurosci. 19:177-181 (1996), that a mutation "might lead to an avalanche of compensatory processes and resulting secondary phenotypical changes." (see page 8 of the Office Action dated May 12, 2005) (emphasis added). Applicant first notes that the Examiner has provided no evidence of the existence of such compensatory processes with respect to the mTMT null mutation and therefore the Examiner has failed to show that it is more likely than not that person skilled in the art would not consider credible any specific and substantial utility asserted by the applicant. See MPEP §2107.II(C)(2). Assuming, *arguendo*, that such compensatory processes do indeed occur, Crawley (1996) Trends Neurosci. 19:181-182 (immediately following the Gerlai article cited above) notes that "[f]rom the point of view of developmental biology, the compensatory process is a fascinating study unto itself. In cases where the knockout mice appear to be phenotypically normal, we have the opportunity to learn a great deal about genetic redundancy and alternative biochemical pathway." Thus, even if such compensatory processes do indeed result from the mTMT null mutation—which is pure speculation at this point and is unlikely given the observed phenotypic abnormalities —then this establishes an additional utility for the claimed mice, namely the study of those compensatory processes.

Applicant hereby submits a Rule 132 declaration from Robert Driscoll, Vice President of Intellectual Property & Legal Affairs of Assignee, Deltagen as evidence of sales and purpose of such use. The declaration states that the claimed mouse has been purchased by at least one pharmaceutical company. This company is one of the ten largest pharmaceutical companies in the world. The declaration further states that the company purchased the claimed mouse for studying gene function and for human therapeutic drug development. Applicant respectfully submits that it runs contrary to common sense to think that one of the world's largest pharmaceutical corporations would purchase the claimed mouse if it thought the mouse had no utility. The test of utility is closely "related to the world of commerce rather than to the realm of philosophy." *Brenner v. Manson*, 383 U.S. 519, 148 U.S.P.Q. 689, 696 (1966). Just as "[p]eople

rarely, if ever, appropriate useless inventions," *Raytheon Co. v. Roper Corp.*, 724 F.2d 951, 959 (1983), large pharmaceutical corporations rarely, if ever, purchase useless inventions. See also *CTS Corp. v. Piher International Corp.* 527 F.2d 95, 105, 188 U.S.P.Q. 419, 428 (7th Cir. 1975) ("[s]ince Piher's trimmer is evidently a commercial success, it seems logical to infer that the subject matter of the invention is useful within the meaning of § 102 [sic]").

Applicant also respectfully directs the Examiner's attention to a recently issued opinion from the Federal Circuit:

> Fisher did not present any evidence showing that agricultural companies have purchased or even expressed any interest in the claimed ESTs. And, it is entirely unclear from the record whether such business entities ever will. Accordingly, while commercial success may support the utility of an invention, it does not do so in this case. See Raytheon Co. v. Roper., 724 F.2d 951, 959 (Fed. Cir. 1983) (stating that proof of a utility may be supported when a claimed invention meets with commercial success).

(*In re Fisher*, 04-1465, 22 (Fed. Cir. 2005)). Unlike Fisher, Applicant has now submitted evidence that the claimed invention has been purchased and delivered to at least one large pharmaceutical company. Applicant respectfully submits that this evidence establishes the utility of the claimed invention.

In view of the arguments made in this office action response, and in the preceeding office action responses, Applicant submits that the invention as claimed has utility. Withdrawal of the 35 U.S.C. § 101 rejection is respectfully requested.

Rejection under 35 U.S.C. § 103

The Examiner has rejected claims 29, 30, and 32 under 35 U.S.C. § 103(a) as being unpatentable over Wong et al. and Smyth et al. in view of Capecchi et al. Applicant has canceled claims 29, 30, and 32 solely to expedite prosecution. Cancellation of these claims does not indicate that the Applicant acquiesces in the Examiner's grounds for rejection, and Applicant reserves the right to later prosecute such claims. Furthermore, the cancellation of these claims does not indicate a surrender of any subject matter encompassed by the claims.

Rejections under 35 U.S.C. § 112

35 U.S.C. § 112, First Paragraph Rejections

The Examiner has rejected claims 24-26, 28-30, 32, and 36-39 under 35 U.S.C. § 112, first paragraph, as failing to comply with the enablement requirement for allegedly containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention. In particular, the Examiner alleges that one would not know how to use the invention; and furthermore, that even if one were to accept that the disclosure satisfies the utility requirement of 35 U.S.C. § 101, the skilled artisan would not be able to use the invention without first engaging in undue experimentation. Applicant respectfully disagrees on both points.

As shown above, the claimed invention has a number of specific, substantial, and well-established utilities *e.g.* the claimed mice may be used in the study of the function of the mTMT gene; in the study of the expression of the mTMT gene; in the study of diseases that result from the disruption of mTMT gene function, and the development of drugs for treating such diseases; and in the study of mTMT as a therapeutic target by mimicking the effect of a mTMT antagonist.

The Examiner argues that the teachings in the application fall short of providing an enabled animal model of any disease. The Examiner states that the specification does not teach whether or not the mice used in the experiments actually comprise a null mutation. Applicant respectfully submits that one skilled in the art would understand based on the Figure 2 of the specification that the targeting construct has generated a null allele of the mTMT gene. The targeting construct deletes bases 164 to base 287 and inserts a LacZ-neo cassette at the site of the deletion. The deletion and the insertion cause a reading frame shift that results in the appearance of stop codons in all three reading frames downstream of the insertion/deletion site. One skilled in the art would immediately understand that such a profound disruption of the mTMT gene will lead to ablation of the gene's function, which is the definition of a null allele.

The Examiner also states that phenotypic differences relative to wild-type mice are not statistically significant. As noted above, Applicant has supplied a 37 C.F.R. § 1.132 declaration showing that the phenotypic differences are statistically significant ($1\text{-}p=0.98$). Accordingly, no further experimentation is required to establish that the phenotypic differences are of sufficient magnitude to be useful as a model.

The Examiner also states that the specification fails to teach what disease state is modeled by the animal. As discussed above, one skilled in the art would be aware of a number of disease states that were known in the art at the time of filing in which mTMT was implicated *e.g.* asthma. In addition, there are numerous other uses of the claimed mouse, referred to above, that would be immediately understood by those skilled in the art and for which the disclosure is fully enabling. For example, the claimed mouse can be used to study expression of mTMT using LacZ staining. The claimed mouse can also be used to study the function of the mTMT gene by, for example, studying the phenotypes resulting from mTMT disruption. Thus, the specification provides an enabling disclosure of multiple ways to use the claimed mouse.

The Examiner cites Gerlai as teaching that knowledge of a phenotype is not sufficient to enable study of gene function because of "compensatory processes." As discussed above, Gerlai indicates that such "compensatory processes" might occur based on a small number of examples involving unrelated genes. Thus, Gerlai does not stand for the general proposition that knowledge of a phenotype is always insufficient to study gene function.

The Examiner cites Crawley as showing that the knockout of the *HEXA* gene in mice produces no ill-effects in mice because the *HEXB* gene can compensate; however mutation in the *HEXA* gene in humans results in Tay-Sachs disease. The Examiner then argues that this indicates that a disease state in mice may not be relevant to any organism other than the mouse itself. Again, the Applicant notes that Examiner is relying on art which shows what might be the case with respect to the mTMT gene based on results obtained for other completely unrelated genes. Again, the Applicant notes that such speculative arguments demonstrate that the Examiner has failed to show the requisite degree of unpredictability.

The Examiner also argues that the art available at the time of filing teaches that correlating any given phenotypic characteristic in a knockout mouse with the functional characteristics of the ablated gene or with a disease state was highly unpredictable. The Examiner cites Wolfer et al. for the proposition that without experimental characterization of the animal, the skilled artisan does not known which phenotypic characteristics are a result of the target gene ablation and which are a result of linkage disequilibrium of genes linked to the target gene. Applicant notes that, according to Wolfer et al.,: "..the possibility exists that an apparent effect of a null mutation could be due to a flanking 129 gene. Generally, the problem is

disregarded because it imposes control strategies deemed costly, and because the statistically expected number of confounding flanking genes is relatively low" (2002, TRENDS in Neuroscience, 25:336-340; page 336) (emphasis added). Thus, a phenotype caused by linked genes is a rare phenomenon. Therefore, contrary to the Examiner's argument, the phenotypic characteristics of the claimed mouse are predictable and are highly likely to result from ablation of the target mTMT gene, rather than from genes in linkage disequilibrium.

The Examiner has repeatedly argued that the use of knockout mice to model a disease—which is only one of the many utilities asserted herein for the claimed mouse—is unpredictable. The Examiner's arguments would seem to suggest that such unpredictability—caused either by linkage disequilibrium, or background, or "compensatory processes"—would lead one skilled in art to regard the creation of knockout mice a futile endeavor. This is clearly not the case. There are literally thousands of knockout mice currently being used around the world to develop drugs, validate gene targets for drugs, and to study gene function. See, for example, Austin et al., Nature Genetics (2004) 36(9):921, 921 (copy previously supplied), where is noted that around 2,500 knockouts have been described in the literature. Clearly, those skilled in the art do not regard the issues of linkage disequilibrium, background, and "compensatory processes" as insurmountable obstacles to using knockout mice. Moreover, assuming, *arguendo*, that these issues must be routinely addressed for every single knocked-out gene—as the Examiner contends—then such routine investigation could not, by definition, constitute undue experimentation. The fact that experimentation may be complex does not necessarily make it undue, if the art typically engages in such experimentation. *In re Certain Limited-Charge Cell Culture Microcarriers*, 221 USPQ 1165, 1174 (Int'l Trade Comm'n 1983), *aff'd. sub nom., Massachusetts Institute of Technology v. A.B. Fortia*, 774 F.2d 1104, 227 USPQ 428 (Fed. Cir. 1985).

35 U.S.C. § 112, Second Paragraph Rejections

The Examiner has rejected claims 24, 28-30, 32, and 36-39, alleging that claims 24 and 30 (from which the remaining rejected claims depend) are indefinite in reciting that the phenotypes exhibited by the animal are decreases or increases in various parameters without specifying a benchmark against which the increase or decrease is established.

As an initial matter, Applicant notes that claim 30 has been cancelled without prejudice; therefore, the rejection will be discussed with respect to claim 24 and the claims dependent therefrom.

The essential inquiry under 35 U.S.C. § 112, Second Paragraph is whether the claims set out and circumscribe a particular subject matter with a reasonable degree of clarity and particularity. The determination is made in view of:

1. the content of the particular application disclosure;
2. the teachings of the prior art; and
3. the claim interpretation that would be given by one possessing the ordinary level of skill in the pertinent art at the time the invention was made.

See MPEP § 2173.02.

Claim 24 recites that the phenotypes exhibited by the transgenic mice are established in comparison to a wild-type control mouse. One skilled in the art would understand that the term "a wild-type control mouse" is a standard term used in the art which refers to a strain, age, and gender matched +/+ mouse. As explained by Crawley (*What's Wrong with My Mouse? Behavioral Phenotyping of Transgenic and Knockout Mice*, Wiley-Liss 2000) (copy of relevant page attached):

> Identified F_1 heterozygote offspring are mated with each other to produce an F_2 generation. Theoretically, the F_2 population with follow the principles of Mendelian segregation, resulting in one-fourth (1/4) homozygous mutants (-/-), one-half (2/4) heterozygotes (+/-), and one-fourth (1/4) homozygous wildtype controls (+/+).

(p. 15)(emphasis added)

Withdrawal of the 35 U.S.C. § 112, Second Paragraph rejection is respectfully requested.

Conclusion

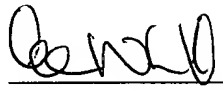
If the Examiner believes a telephone conference would advance the prosecution of this application, the Examiner is invited to telephone the undersigned at the below-listed telephone number.

This constitutes a request for any needed extension of time under 37 C.F.R. § 1.136(a) and an authorization to charge all fees therefore to deposit account No. 502775 if not otherwise specifically requested.

The Commissioner is hereby authorized to charge any required fees not included, or any deficiency of fees submitted herewith, or credit any overpayment to Deposit Account No. 502775.

Respectfully submitted,

9/12/2005
Date



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Industrializing breakthrough discovery

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Predicting drug action using mouse knockouts was pioneered by Lexicon; five years later the full potential of gene knockout technology is beginning to be realized. In combination with comprehensive physiological analysis, the technology delivers novel, *in vivo*-validated targets with the potential for the discovery of breakthrough therapeutics.

Innovation in the pharmaceutical industry depends on breakthrough biological discoveries that reveal new targets for therapeutic intervention. These new targets must provide potent new mechanisms of action to block disease by creating favorable alterations in physiology without undesirable side effects. *In vivo* methods of target validation using gene knockouts have revealed truly rare and valuable targets. This fact stands in direct contrast to the popular myth that the human genome contains thousands of viable drug targets. It seems that a simple truth has been all but forgotten in the pursuit of ultra-high-throughput drug discovery: it is the quality of new targets not the quantity that holds promise for replenishing the pharmaceutical industry's drug discovery pipeline.

There is plenty of evidence that more is not necessarily better. The pharmaceutical industry spends $30 billion each year on research and development - three times more than a decade ago - yet the number of new drugs coming to market has not increased. The industry's product innovation bottleneck is especially critical since $38.6 billion in brand name drugs will be coming off patent over the course of the next three years, creating a market void that drug makers are not prepared to fill.

Physiology must guide discovery

To replenish product pipelines, the industry is looking to biotechnology companies to accelerate the identification and validation of new targets. In order to discover which genes among thousands encode breakthrough targets, industry scientists must conduct rigorous physiological assessments to determine which targets to eliminate and which to pursue. Only those targets that demonstrate the potential to maximize therapeutic effects and minimize side effects should be pursued, thereby reducing the failure rate and increasing the overall efficiency of the drug discovery process.

Since a therapeutic alteration in physiology is the desired endpoint of drug discovery, overly reductionist approaches that ignore the complexity of mammalian physiology are inevitably doomed to failure. Computer modeling, DNA microarrays, proteomics and lower model organisms cannot encompass the complexity of mammalian physiology and may actually distract researchers from a more productive pathway to discovery. Even human genetic studies may be problematic, since they are more likely to reveal genes that cause disease rather than drug targets for future cures.

Just as drugs must act within the context of physiology, novel drug targets must be validated within the context of mammalian physiology before precious resources are expended to develop drugs. Grounding genomics in the discipline of physiology can increase success rates, enhance product pipelines and create safer and superior therapeutics, as well as reduce the enormous amount of time and capital expended for the discovery and development of a drug. Those companies who are equipped to rapidly and effectively integrate physiological information into the target selection process will dominate the next generation of successful drug discoveries.

Of knockout mice and men

After a decade of using mouse knockouts, the data on their predictive power in drug discovery is irrefutable. The top 100 selling drugs in 2001 are directed only to 29 drug targets, many with multiple agents addressing the same target. Of these 29 targets, 23 have been knocked out and in every case the knockout mouse was highly predictive as to the on-target effects and side effects of the associated drugs. These observations lay to rest early theoretical concerns regarding the reliability of the mouse knockout technology to recapitulate actions of drugs in mammalian model systems. The recent near completion of the genomic sequence of mouse and man, now available through either public or private DNA sequence databases, has confirmed the high rate of genomic similarity between the two organisms. Indeed, many decades of research have proved the mouse to be an invaluable tool for the evaluation of biological processes relevant to human disease, including immunology, oncology, neurobiology, cardiovascular biology, obesity and many others. Well-established parallels exist between humans and mice on cellular, biochemical and physiological levels.

Industrializing discovery

At Lexicon Genetics mouse knockouts are guiding researchers to discover new therapeutic agents which represent the best

physiologic switches in the genome for the treatment of disease. This has required the industrialization of gene targeting, gene trapping and mouse embryonic stem cell technologies, as well as the build-up of significant scientific infrastructure. This infrastructure will allow the company to analyze 5000 genes as mouse knockouts in its Genome5000 program over the next five years. Efforts are concentrated on the unknown function of known gene families for which therapeutic agents can be developed through small molecule chemistry, antibody or therapeutic protein development. These gene families include G protein-coupled receptors, kinases, proteases, ion channels, secreted proteins, transporters and other key enzyme classes. Gene targeting by homologous recombination combined with gene trapping maximizes both selectivity and throughput for large-scale, *in vivo* target validation.

"It seems that a simple truth has been all but forgotten in the pursuit of ultra-high-throughput drug discovery: it is the quality of new targets not the quantity that holds promise for replenishing the pharmaceutical industry's drug discovery pipeline."

The company has deployed a comprehensive, *in vivo* analysis of candidate drug targets that has been modeled after clinical evaluation. Genes analyzed in this way are subject to a superior level of *in vivo* analysis, including physiological function and potential disease indication, providing a robust pipeline of high-value targets. This approach has already proved successful in extracting vital information about the potential medical utility of several new targets in atherosclerosis, diabetes, obesity and CNS disease, among others. Lexicon's physiological analysis utilizes a wide range of the latest medical technologies, including intensive analytical procedures such as the CAT scan for organ system visualization, dual energy X-ray absorptiometry for measurement of percentage fat and lean body mass and bone mineral density, functional magnetic resonance imaging, which allows *in vivo* neurochemical and cardiac analysis, clinical blood and urine chemistries, complete blood cell counts, fluorescent-activated cell sorting, cell-cycle analysis and neurobehavioral testing. Histopathological and gene expression surveys of 55 tissues provide cellular and gene expression data for additional information. Disease challenge models may also be used when indicated to maintain a

Computer modeling, DNA microarrays, proteomics and lower model organisms cannot encompass the complexity of mammalian physiology and may actually distract researchers from a more productive pathway to discovery.

high degree of sensitivity, enabling the detection of subtle phenotypes that may be of significant medical value.

The phenotype derived from the knockout of a specific gene reveals both the potential therapeutic value as well as other target-specific side effects that may be anticipated for a small molecule inhibitor of that target. For instance, a target may display therapeutic potential in inflammation, but might also be critical for renal function. Without a mammalian knockout model, these deleterious target-specific side effects might not be observed until after significant amounts of time and resources have been spent on developing small-molecule compounds and testing them in preclinical or clinical development. When a drug produces a deleterious effect that was not observed in the knockout animal, it suggests that further optimization of the compound's specificity is worthwhile. The ability to produce strong preclinical data to support efficacy and lack of deleterious side effects for a novel target and corresponding lead compound further legitimizes the value of a drug discovery program and provides confidence to move ahead aggressively in development.

Predicting breakthrough therapeutics

Gene knockouts can be viewed as modeling the biological mechanism of drug action by presaging the activity of highly specific antagonists *in vivo*. This information is critical when making decisions regarding target prioritization for a drug discovery enterprise. Since knockout mice have been shown to model drug activity, they provide an unprecedented level of predictive power over the drug discovery process and can be extremely valuable to the pharmaceutical and biotechnology industries. With the effective use of mouse knockout technology, expensive drug discovery activities such as high-throughput screening, medicinal chemistry, preclinical research and clinical trials can be focused on the drug targets that are most likely to lead to breakthrough therapeutics.

Hypothesis-driven gene targeting and gene trapping place physiology and therapeutic potential at the forefront of the drug discovery process and will provide primary data on the physiological function of virtually all members of 'drugable' gene families over the next few years. However, the full power of knockout mouse technology can only be realized when the predictive nature of knockout mouse phenotypes is applied early in the drug discovery process. The combination of mouse gene knockout technology and comprehensive physiological analysis will provide the pharmaceutical industry with novel, *in vivo*-validated targets with clear potential for the discovery of breakthrough therapeutics.

> ***"The top 100 selling drugs in 2001 are only directed to some 29 drug targets, many with multiple agents addressing the same target. Of these 29 targets, 23 have been knocked out and in every case the knockout mouse was highly informative as to the on-target effects and side effects of the associated drugs."***

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FURTHER READING

Firn A, Griffith (2002) **Big pharma hopes to get by with some help from old friends.** *Financial Times* May 01.

(2002) **Worldwide functional genomics market expected to reach $2 billion in 2007.** *PR Newswire: Front Line Strategic Consulting* April 18.

Meeting preview

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Characterization of Human γ-Tryptases, Novel Members of the Chromosome 16p Mast Cell Tryptase and Prostasin Gene Families[1]

George H. Caughey,[2] Wilfred W. Raymond, John L. Blount, Leola W.-T. Hau, Michele Pallaoro, Paul J. Wolters, and George M. Verghese

Previously, this laboratory identified clusters of α-, β-, and mast cell protease-7-like tryptase genes on human chromosome 16p13.3. The present work characterizes adjacent genes encoding novel serine proteases, termed γ-tryptases, and generates a refined map of the multitryptase locus. Each γ gene lies between an α1H Ca^{2+} channel gene (*CACNA1H*) and a βII- or βIII-tryptase gene and is ~30 kb from polymorphic minisatellite MS205. The tryptase locus also contains at least four tryptase-like pseudogenes, including mastin, a gene expressed in dogs but not in humans. Genomic DNA blotting results suggest that γI- and γII-tryptases are alleles at the same site. βII- and βIII-tryptases appear to be alleles at a neighboring site, and αII- and βI-tryptases appear to be alleles at a third site. γ-Tryptases are transcribed in lung, intestine, and in several other tissues and in a mast cell line (HMC-1) that also expresses γ-tryptase protein. Immunohistochemical analysis suggests that γ-tryptase is expressed by airway mast cells. γ-Tryptase catalytic domains are ~48% identical with those of known mast cell tryptases and possess mouse homologues. We predict that γ-tryptases are glycosylated oligomers with tryptic substrate specificity and a distinct mode of activation. A feature not found in described tryptases is a C-terminal hydrophobic domain, which may be a membrane anchor. Although the catalytic domains contain tryptase-like features, the hydrophobic segment and intron-exon organization are more closely related to another recently described protease, prostasin. In summary, this work describes γ-tryptases, which are novel members of chromosome 16p tryptase/prostasin gene families. Their unique features suggest possibly novel functions. *The Journal of Immunology,* **2000, 164: 6566–6575.**

Tryptases are expressed by most human mast cells and are abundant, comprising as much as 25% of cell protein (1). They are trypsin-like proteases secreted as active, heparin-bound oligomers that resist inactivation by plasma antiproteases (2). Extracellular targets include neuropeptides, procoagulant proteins, urokinase, stromelysin, and proteinase-activated receptor-2 (3–7).

Several studies suggest roles for tryptases in allergic airway disease. Lavage tryptase levels rise in asthmatics following endobronchial allergen challenge (8). In dog and human bronchi, tryptases increase histamine-induced bronchoconstriction (9, 10) and also may promote constriction by degrading vasoactive intestinal peptide (4). By stimulating airway smooth muscle cell (11) and fibroblast growth (12, 13), as well as chemotaxis and collagen synthesis (14, 15), they may contribute to muscle hypertrophy and subepithelial fibrosis in asthma. The hypothesis that tryptases worsen asthma is buttressed by reports that tryptase inhibitors block allergic bronchoconstriction and eosinophilic inflammation in sheep (16) and, in the first human trials of tryptase inhibitors, reduce asthmatic responses to inhaled allergen in asthmatics (17).

Known human tryptases divide into two groups, α and β. α-Tryptase is the major circulating isoform and may be the main type expressed by basophils (18, 19). β-Tryptases appear to be the major type stored in secretory granules and are the major form isolated from lung extracts and mast cells purified from lung and skin (19). Four different human cDNAs (α, βI, βII, and βIII) have been isolated from lung and skin mRNA (20–22). β-Tryptases are 98–99% identical in amino acid sequence. α-Tryptases are less closely related (αI is 91% identical with βI). Our laboratory reported recently that αII, βI, βII, and βIII genes are clustered on chromosome 16p13.3 along with genes encoding novel tryptases, the fifth exon of which is related to the mouse tryptase, mouse mast cell protease (mMCP)-7[3] (23). The intron-exon organization of tryptase genes is unique, suggesting that tryptases are a distinct and perhaps ancient branch of the trypsin clan of serine peptidases.

In mice, tryptases mMCP-6 and -7 have been identified (24, 25). Structurally, human α/β-tryptases are more closely related to each other than to mMCP-6 and -7, which are much more different from each other than any combination of known human tryptases. This suggests that mMCP-6 and -7 are not the equivalents, respectively, of α- and β-tryptases and that ancestors of α/β-tryptases diverged from each other after the point when known mouse and human tryptases shared a common ancestor (23, 26). In dogs, our laboratory characterized a mast cell protease termed mastin, a relative of tryptase sufficiently different from known tryptases that it forms a separate branch of the tree (27–29). No expressed human or mouse homologue has been identified.

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[3] Abbreviations used in this paper: mMCP, mouse mast cell protease; EST, expressed sequence tag; HTGS, high throughput genome sequence; BAC, bacterial artificial chromosome.

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Here we demonstrate expression of novel human tryptases, termed γ because they are distinct from α, β, and other human tryptases. We also present a refined map of α/β genes and identify a gene encoding a human homologue of dog mastin.

Materials and Methods

Database screening

Human βI-tryptase cDNA (22) and gene sequence (23) and Basic Local Alignment Search Tool (www.ncbi.nlm.nih.gov) algorithms were used to query expressed sequence tag (EST) and high throughput genome sequence (HTGS) in GenBank. Predicted novel human cDNAs were used to query GenBank's mouse EST database to identify mouse homologues.

Cell culture

Cells from the human mast cell line HMC-1 (5C6 subclone, kindly provided by Dr. Beate M. Henz) were cultured as described (30). HMC-1 cells express active βI-tryptase but not α-tryptase (19, 30–32).

Amplification and cloning of cDNAs

cDNA predicted from EST and genomic sequence was used to design PCR primer pairs, which then were used to screen cDNAs prepared from a spectrum of human tissues for expression of γ-tryptases. Sequencing of amplimers allowed us to prove the identity of the PCR-derived bands, to confirm exon sequences predicted from genomic and EST DNA, and to confirm intron-exon splice site predictions. Amplified fragments of γ-tryptase cDNA from human lung and from HMC-1 5C6 cells were subcloned into pCR2.1 (Invitrogen, Carlsbad, CA) for further sequencing and for generation of specific probes to use in blotting studies. Bands containing amplimers from colon, small intestine and testis were sequenced directly.

DNA and protein sequence comparisons

DNA sequencing was conducted by University of California at San Francisco's Biomolecular Resource Center using standard dideoxy techniques with fluorescent dye terminators. Multiple sequence alignments, matrix analyses, and dendrograms were generated using GeneWorks software (Oxford Molecular, Campbell, CA).

Chromosomal localization and screening of bacterial artificial chromosomes (BACs)

BAC clones 324 and 48 previously were identified and localized to chromosome 16p13.3 as described (23). In the current study, γ-tryptase genes were detected in these BACs by PCR of gene-specific fragments and by DNA blotting using the general approaches we described previously in connection with these BACs (23). BAC fragments generated by digestion with *Hin*dIII were subcloned. Genes were mapped to specific sites in BAC λ subclones by blotting of electrophoresed DNA restriction digests and by sequencing of PCR amplimers, BAC ends, and portions of selected λ subclones.

mRNA and DNA blotting

mRNA extracted and purified from cultured HMC-1 5C6 cells was electrophoresed in agarose and blotted to nylon membranes. These blots, along with blots of mRNA from a range of human tissues (Invitrogen), were hybridized with radiolabeled cDNA probes corresponding to protein-coding exons. BAC and full human genomic DNA were digested with restriction endonucleases, size-fractionated in agarose, then blotted, baked, and prehybridized as described (23). The resulting blots were probed with a radiolabeled 1100-bp fragment of the BAC 48 γ-tryptase gene bracketing the internal *Not*I site, or with labeled subclones of this fragment lying on each side of the *Not*I site, then subjected to autoradiography.

Ab generation, immunoblotting, and immunohistochemistry

Polyclonal Abs recognizing human γ-tryptase were raised in chickens immunized with a keyhole limpet hemocyanin-conjugated synthetic peptide (CRRDYPGPGGSILQP) corresponding to residues 192–206 of human prepro-γ-tryptase. Conjugations and immunizations were conducted by AnaSpec (San Jose, CA). In preparation for immunoblotting, HMC-1 5C6 cell proteins were extracted into detergent solution (4% SDS in 0.2 M DTT, 20% glycerol, 0.125 M Tris, pH 6.8). Extracts were electrophoresed in 12.5% SDS-polyacrylamide gels and electroblotted to polyvinylidene difluoride membranes, which were preincubated with 0.3% Tween-20 in 50 mM Tris (pH 7.2) and 0.5 M NaCl, then hybridized with 1:1000 dilutions of chicken antiserum for 1 h in the same buffer. Bound Abs were detected with goat anti-chicken IgG-alkaline phosphatase (1:5000) and Fast Red TR/Naphthol AS-MX Phosphate (Sigma, St. Louis, MO). Control blots were incubated with preimmune chicken serum or with secondary Ab.

For immunohistochemical analysis using the same anti-γ-tryptase antiserum, samples of large airways were obtained (with approval of the University of California at San Francisco Committee on Human Research) from patients undergoing lung transplantation for cystic fibrosis. Five-micrometer airway cryosections placed onto glass slides were equilibrated in 0.3% H_2O_2 and 90% methanol for 10 min, washed with PBS, then incubated for 15 min with blocking solution (PBS containing 5% dehydrated milk, 3% nonimmune goat serum, 0.1% Triton X-100, and 1% glycine) at 18°C. Blocking solution was removed and tissues probed either with a 1:100 dilution of chicken nonimmune serum or anti-γ-tryptase antiserum overnight at 4°C. Tissues were then washed in PBS plus 0.05% Tween-20, incubated with a 1:200 dilution of alkaline phosphatase-conjugated goat anti-chicken IgY Ab (Promega, Madison, WI) for 10 min at 18°C, then washed again. Bound alkaline phosphatase was detected using the substrate 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium (Zymed Laboratories, South San Francisco, CA).

Molecular modeling

We constructed a homology-model of the γI-tryptase catalytic domain with assistance of an automated protein modeling tool and server (Swiss PDB Viewer and Swiss-Model, respectively) (33). The pro- and C-terminal hydrophobic sequences were excluded from the model. X-ray diffraction-derived coordinates of human βII-tryptase (1AOL) (34) served as template for the model, which was optimized by idealizing bond geometry and removing unfavorable nonbonded contacts.

Results and Discussion

Identification of γ-tryptase cDNAs and prediction of amino acid sequence

cDNAs encoding parts of human γ-tryptase were obtained initially by screening EST databases using known tryptases as query sequences. Because these sequences are partial and disagree in certain areas of overlap, PCR primers based on EST sequence were used to amplify more complete cDNAs from human lung cDNA. The full amino acid sequence of 321 amino acids deduced from a combination of PCR-derived lung cDNA and EST sequence is identical with that predicted for gene-derived γI-tryptase in Fig. 1, which compares gene-derived prepro-γ-tryptase primary sequences with those of related serine proteases. The γI and γII structures aligned in Fig. 1 represent amino acid sequences predicted from separate BAC genes (see below). The predicted m.w. of prepro-γI-tryptase is 33,689 without glycosylation or other post-translational modification. Based on database searches and computer-assisted multiple sequence alignments and phylogenetic comparisons, the γI-tryptase catalytic domain amino acid sequence is most similar to that of known mast cell tryptases, exhibiting 47% identity to βII-tryptase. Some structural features, e.g., LPPPY (residues 173–177), are particularly tryptase-like. Other features are common to all active serine proteases with tryptic specificity, such as "catalytic triad" residues His^{78} (His^{57}, by standard chymotrypsinogen numbering), Asp^{125} (Asp^{102}, standard numbering), and Ser^{222} (Ser^{195}, standard numbering), and also residue Asp^{216} (Asp^{189}, standard numbering), which is the prime determinant of specificity for Arg or Lys on the N-terminal side of the scissile bond of peptide substrates. These features, along with the clustering of γ-tryptase genes with known tryptase genes and evidence of mast cell expression, are the basis of labeling these enzymes tryptases. However, the catalytic domains are related almost as closely to the recently described proteases prostasin (35) and testisin (36). Indeed, when full preprosequence and gene structures (see below) are compared, γ-tryptase appears to be more similar to prostasin and testisin than to α/β-tryptases. Features shared with prostasin and testisin include a propeptide ending in Arg and a predicted C-terminal membrane anchor, neither of which are

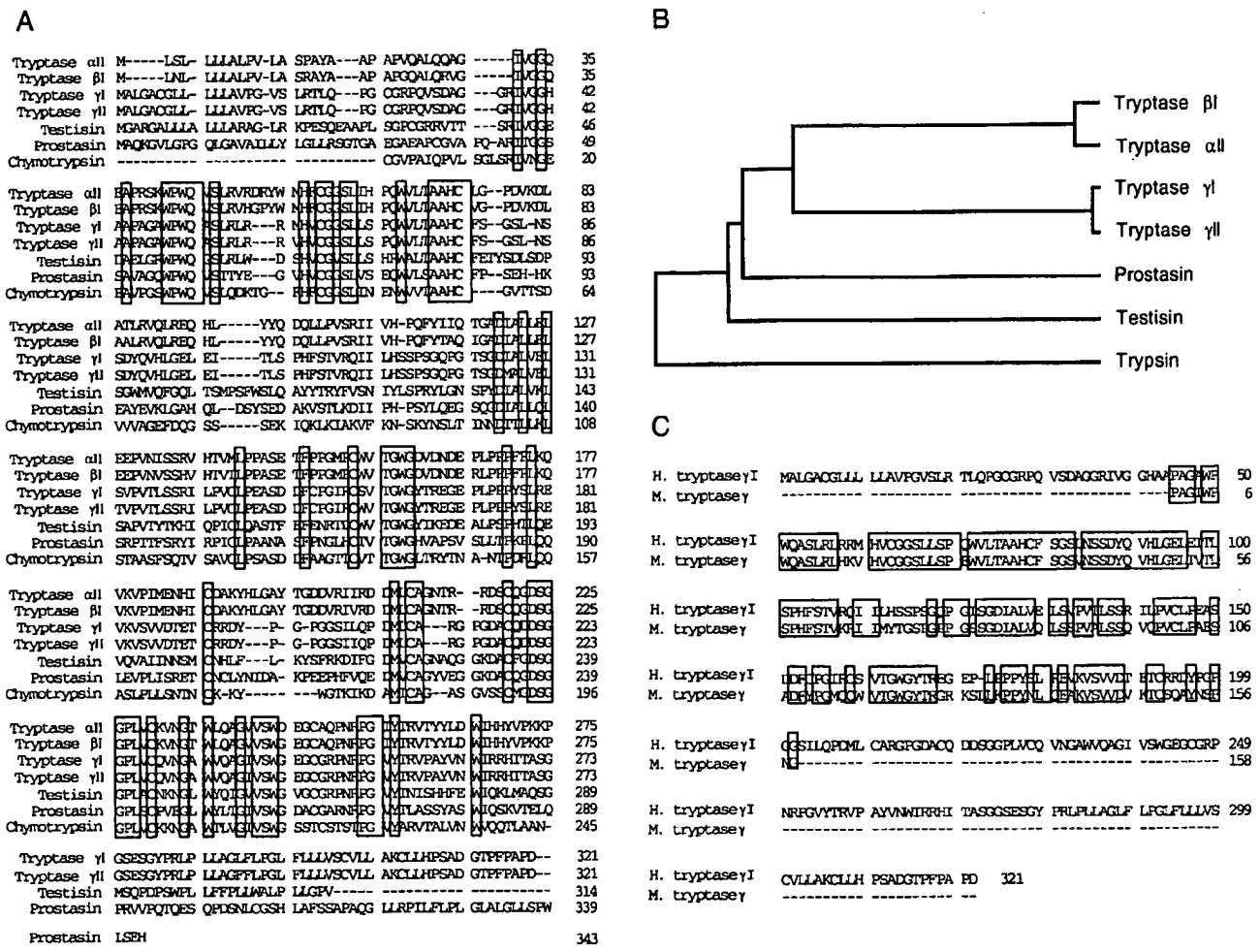

FIGURE 1. Alignment and phylogenetic comparison of γ-tryptase with related serine proteases. *A*, Computer-aligned preprosequences of αII-tryptase, βI-tryptase, γ-tryptases, testisin, and prostasin. Chymotrypsin is also included to show the positions of key residues numbered according to standard serine protease numbering (Ser^{195}, etc.), which is based on the sequence of chymotrypsinogen. Residues that are identical in all seven proteases are enclosed in boxes. *B*, A dendrogram developed from the same sequences (catalytic domains only) exchanging chymotrypsin for trypsin, which is more closely related to tryptases in substrate specificity. The length of each branch is proportional to the percentage of sequence mismatches between pairs or groups of sequences. The results suggest that the catalytic domains of γ-tryptases are more closely related to α/β-tryptases than to testisin and prostasin. *C*, Alignment of γI-tryptase amino acid sequence with the partial sequence of a putative mouse homologue deduced from ESTs. The high degree of correspondence between the two sequences suggests that mice possess one or more homologues of human γ-tryptases.

present in α/β-tryptases, and a gene organization more similar to that of prostasin than of previously described tryptases (see below).

Termination of the putative propeptide in a basic residue suggests that pro-γ-tryptase is activated directly from its catalytically inactive zymogen form by a tryptic protease, which will sever the propeptide from the catalytic domain, allowing it to adopt an enzymatically active conformation. This mode of activation would differ from that of human β-tryptases, which lack a terminal propeptide basic residue and are activated in a two-step process involving tryptase and dipeptidyl peptidase I (37). Mature, active γ-tryptase, unlike known tryptases, may be a two-chain protein, with part of the propeptide remaining covalently attached via a disulfide linkage involving Cys^{26} (Cys^{1} in chymotrypsinogen) by analogy to the propeptide-catalytic domain linkage in activated chymotrypsin (38). Prostasin and testisin also contain this predicted linkage. It is possible that the γ-tryptase propeptide will be removed through the action of exopeptidases such as dipeptidyl peptidase I, which is highly expressed in mast cell secretory granules (39). Based on a consensus site at Asn^{85}, mature γ-tryptases may be N-glycosylated. The mature protein is likely to be an active protease with tryptic specificity, based on conservation of critical triad residues and of specificity-determining Asp^{216} (Asp^{189}, by chymotrypsinogen numbering), as noted above. Due to conservation of the LPPPY sequence found to form noncovalent contacts between subunits in the βII-tryptase tetramer (34), γ-tryptase may form oligomers of catalytically active units. However, γ-tryptase lacks the pair of Tyr residues that also are predicted to promote LPPPY-mediated oligomerization. Therefore, it is not yet possible to predict with certainty whether γ-tryptase will oligomerize, like all heretofore-characterized mast cell tryptases and close relatives, such as mastin (29).

γ-Tryptases I and II contain a C-terminal extension with a highly hydrophobic segment long enough to form a single-pass transmembrane helix, as shown by the hydropathy plot in Fig. 2. This predicts that the catalytic domain will be C-terminally membrane anchored. Within the endoplasmic reticulum, Golgi, and secretory granules, the γ-tryptase catalytic domain should lie in the lumen. If γ-tryptase traffics to the cell surface, then the catalytic domain will lie on the extracellular surface. The hydropathy analysis predicts a small cytoplasmic tail of uncertain significance. The

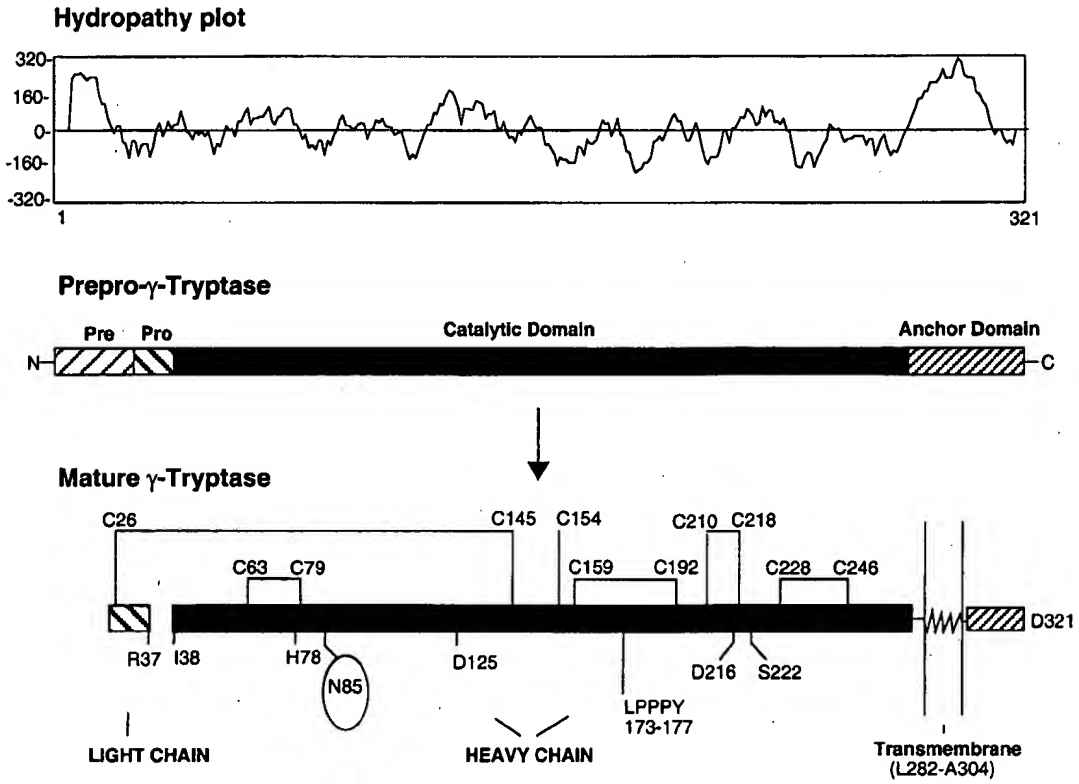

FIGURE 2. Predicted posttranslational modifications of human γ-tryptase. The *top panel* depicts a Kyte-Doolittle analysis of the 321-residue preproenzyme, plotting residue number (*x*-axis) vs hydropathy index (*y*-axis). The results predict a catalytic domain flanked by hydrophobic sequences, including a classic signal prepeptide on the N-terminal side (residues 1–19) and a membrane-anchoring segment on the C-terminal side, as depicted in the *middle panel*. Removal of the prepeptide by signal peptidase leaves a propeptide, which is disconnected from the catalytic domain by hydrolysis at Arg^{37} to generate the active enzyme. As shown in the *bottom panel*, we predict that active γ-tryptases, unlike known tryptases, are two-chain proteins in their mature forms, with a portion of the prosequence remaining attached to the catalytic domain via Cys^{26}. Other disulfide pairs are as indicated, by analogy to βII-tryptase and trypsin. We predict one unpaired cysteine at residue 154. γ-Tryptase may be N-glycosylated at a single site, Asn^{85}. We predict that the mature protein will be catalytically active based on conservation of critical "catalytic triad" residues His^{78}, Asp^{125}, and Ser^{222} that are essential for enzymatic function of all serine proteases and that the mature enzyme will hydrolyze its targets after basic residues based on conservation of Asp^{216}, which is the major determinant of Arg/Lys specificity in known tryptic serine proteases. Based on conservation of the LPPPY sequence found to form noncovalent contacts between subunits in the βII-tryptase tetramer, γ-tryptases may form oligomers of catalytically active units.

10 residues on the N-terminal side of the hydrophobic tail embody features typical of GPI-anchored proteins (40). Possibly, the transmembrane sequence in mature γ-tryptase is severed in the endoplasmic reticulum and replaced with a GPI membrane anchor. If this is the case, γ-tryptase could be released in a soluble form by GPI-specific phospholipases. The predicted m.w. of the unglycosylated γI-tryptase catalytic domain plus C-terminal extension is 30,063. Without the C-terminal extension, the predicted m.w. is ~25,000. The deduced amino acid sequences of γ-tryptases I and II are 98–99% identical with that of recently described "transmembrane tryptase" (41).

Using our human γ-tryptase cDNA, we identified mouse ESTs (e.g., AI466869 and AA266560) collectively encoding 158 residues of contiguous sequence in the catalytic domain of a related mouse enzyme. Based on an amino acid identity of ~74% compared with human γ-tryptase in regions of overlap, the mouse products are likely to be orthologs of human γ-tryptases. Whether they are products of more than one gene remains to be determined. This level of identity is similar to that between human γ-tryptases and the mouse tryptases mMCP-6 and -7 (23). Among features conserved between mouse and human enzymes are catalytic triad residues (His and Asp but not Ser are covered by the partial mouse sequence), the consensus N-glycosylation site, and five of six cysteines.

Tissue expression of γ-tryptase mRNA

As shown by the results of PCR in Fig. 3, we identify transcription of human γ-tryptase in multiple tissues, including lung and small intestine. However, several tissues screened using the same conditions yield no signal or faint signals, suggesting tissue-specific differences in levels of γ-tryptase transcription. Tissues expressing no detectable γ-tryptase mRNA include peripheral blood leukocytes and thymus, a finding that suggests that γ-tryptases are not highly expressed in circulating immune cells. In contrast, γ-tryptase mRNA is abundant in the 5C6 subclone of HMC-1 cells, which also express active βI-tryptase (19, 30–32). Sequencing of PCR-generated amplimers from HMC-1 cells reveals the same sequence predicted from amplimers of human lung cDNA. These sequences, which cover 70% of the protein sequence predicted from BAC genes, is 99.1–97.8% identical with that of γI- and γII-tryptase, respectively (see Fig. 1) in 224 residues of overlap. Blotting of electrophoresed human mRNA from a number of tissues does not yield strong hybridization signals (not shown) with γ-tryptase probes, suggesting that overall levels of γ-tryptase transcript are low (although detectable by PCR), perhaps because γ-tryptase is expressed in a small subpopulation of cells in the tissues surveyed. However, γ-tryptase mRNA is detected by standard blotting of mRNA from the 5C6 subclone of HMC-1 cells

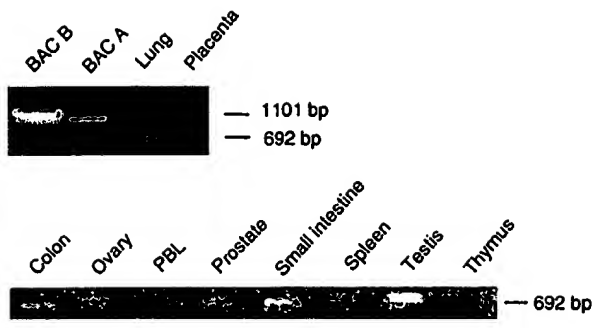

FIGURE 3. Detection of γ-tryptase gene transcription by PCR. A primer pair (5′-AGATCACTTTGTCTCCCCACTTCTC and 5′-TTG GATTCCTGCCATCAGTCAG, based on exons 4 and 6, respectively) was designed to bracket the insertion sites of introns 4 and 5 (see Fig. 6). The predicted length of an amplimer of genomic DNA is 1101 bp and of intronless cDNA is 692 bp. PCR was conducted using AdvanTaq Plus (Clontech, Palo Alto, CA) or PFU Turbo (Stratagene, La Jolla, CA) DNA polymerase as follows: after incubating for 1 min at 95°C, samples were subjected to 35 cycles of denaturation at 95°C for 0.5 min, annealing at 61°C for 1 min, and extension at 72°C for 1 min. Results of PCR using these primers are shown in these ethidium-stained agarose gels. cDNA from a variety of human tissues yields bands of the predicted size. Of tissues surveyed, strong 692-bp bands are obtained from lung, ovary, prostate, small intestine, and testis. A strong 692-bp band also is obtained from HMC-1 cell cDNA (not shown). A faint band is obtained from placenta, colon, and spleen but none from PBL or thymus. Amplimers obtained from lung, small intestine, colon, small intestine, and HMC-1 cells were sequenced. All sequences were nearly identical with the sequence predicted from BAC γ-tryptase exons. The same primers generate bands of ~1100 bp from BAC 324 and 48 as shown. These BAC amplimers yield the expected γ-tryptase sequence (plus introns) upon direct sequencing.

(not shown). The identification of γ-tryptase mRNA in a mast cell line suggests that mast cells may be among the subset of cells expressing γ-tryptases.

Localization of γ-tryptase genes

Previously, this laboratory identified two BAC clones containing clustered α/β-tryptase genes localizing to chromosome 16p13.3 (23). Overlapping portions of these BACs are homologous but not identical. Hypothesizing that additional tryptase-like genes lie in these two BACs (324 and 48 in Fig. 4), we screened both BACs for the presence of γ-tryptase genes via PCR using EST- and cDNA-based primers. Each BAC yields a strong band of amplimers, one of which was subcloned and sequenced, confirming the presence of genomic sequence containing the exon sequence predicted by γ-tryptase ESTs. To localize γ-tryptase genes within each BAC, radiolabeled probes based on cloned fragments of γ-tryptase were hybridized with BAC DNA digested singly and in combination with *Hin*dIII, *Not*I, and *Eco*RI. In each BAC clone, the results localized a γ-tryptase gene to a particular *Hin*dIII fragment bracketing a *Not*I site splitting the gene, as shown in Fig. 4. The *Not*I site in the two γ-tryptase genes was localized by sequencing genomic fragments generated by PCR. The transcriptional orientation of each γ-tryptase gene was determined by DNA blotting using radiolabeled probes derived from the 5′ side of the established *Not*I site. The γI-tryptase gene on BAC 324 lies in a region covered by a subcloned *Hin*dIII fragment (λ324D) found previously to contain the βII gene (23). On BAC 48, the nearest tryptase neighbor is the βIII gene, which resides on an adjacent *Hin*dIII fragment (λ48 M) rather than on the same *Hin*dIII fragment due to the presence of a *Hin*dIII site not present in the otherwise homologous region of BAC 324. As shown in Fig. 5, DNA blots of *Hin*dIII-digested BAC 324, BAC 48, and full genomic DNA reveal that the *Hin*dIII isoforms predicted by the two BACs both are present in some but not all individuals, suggesting that γI and γII are alleles of each other. By inference, based on the homologies between BAC 324 and 48 depicted in Fig. 4, other allelic pairs of tryptases are βII/βIII, αII/βI, and mMCP-7-like I/II, respectively.

Based on examination of γ-tryptase ESTs and sequenced portions of the BAC-derived genes, the nearest neighboring gene on the 3′ flank is an αIH T-type Ca^{2+} channel gene identical or closely related to *CACNA1H*. In the case of the BAC 324 γI-tryptase gene, the 3′ untranslated region possibly overlaps with that of *CACNA1H*, which is in the opposite transcriptional orientation, as depicted in Fig. 4. *CACNA1H* has been localized to chromosome 16p13.3 by fluorescent in situ hybridization (42). A mouse homologue (*Cacna1h*) has been mapped to chromosome 17 in a region that is homologous with a portion of human chromosome 16p13.3 (42). This syntenic region also harbors two mouse tryptase genes, *Mcpt6* and *Mcpt7* (43). At least two genomic fragments encoding parts of *CACNA1H* were identified in database searches. One of these (GenBank AL031703.9) aligns with one end of BAC 324 based on shared restriction patterns and a segment of near-identity with BAC 324's sequenced T7 end, as shown in Fig. 4, allowing extension of the γI-tryptase locus beyond the BAC terminus. A second GenBank sequence (fragment 10 of unfinished chromosome 16-derived contig AL031715.1) contains additional *CACNA1H* -related exons and aligns elsewhere on BAC 324, as shown in Fig. 4. The 5′ end of *CACNA1H* predicted by cDNA cloning was not found in the AL031703.9 sequence; therefore, the extended BAC 324 sequence in Fig. 4 does not include the entire gene. However, AL031703.9 contains the well-known minisatellite MS205 (also known as DS16S309), which is highly polymorphic and localizes to 16p13.3, ~1.2 mb from 16pter (44). MS205, which has been used to explore the origin of human populations (45), is ~30 kb from the γI-tryptase gene.

BAC 48, which contains the γII-tryptase gene, also contains an αIH T-type Ca^{2+} channel gene, based on sequencing of the γII gene itself (see below) and of the BAC insert's SP6 end (see Fig. 4), which contains part of a late *CACNA1H*-like exon in the sequenced portion. This finding strengthens the finding of homology between the two BACs based on restriction mapping and alignment of tryptase genes. BAC 48's T7 end extends beyond the area of homology with BAC 324. The sequence of the T7 end is almost identical with that of fragment 5 of a partially sequenced contig (GenBank AL031711.16), which encodes three tryptase-like genes, MP-2, MP-7, and mastin, as shown in Fig. 4. All three genes have multiple flaws, e.g., premature stop codons, faulty intron-exon junctions (not shown). None have closely related ESTs. Thus, they are likely to be pseudogenes. The mastin gene, whose exons are most closely related to the tryptase-like gene mastin characterized in dogs, is discussed in more detail below.

γ-Tryptase gene sequence and organization

The BAC 324 and 48 γ-tryptase genes, termed γI- and γII-tryptase, respectively, were fully sequenced to reveal their relationship to the cDNAs in EST databases and in DNA amplified by PCR. The GenBank accession numbers of the γI and γII genes are AF191031 and AF195508, respectively. In these genes, intron-exon splice junctions were identified using open reading frames and cDNA alignments by application of the "GT. . . AG" rule for initiating and ending introns and by referring to patterns of intron phase and placement in known tryptases and in other serine protease genes, as described (23). As revealed in Fig. 5, we find that fragment 12 of the unfinished chromosome 16-derived contig AL031715.1 deposited in the HTGS database contains the partial

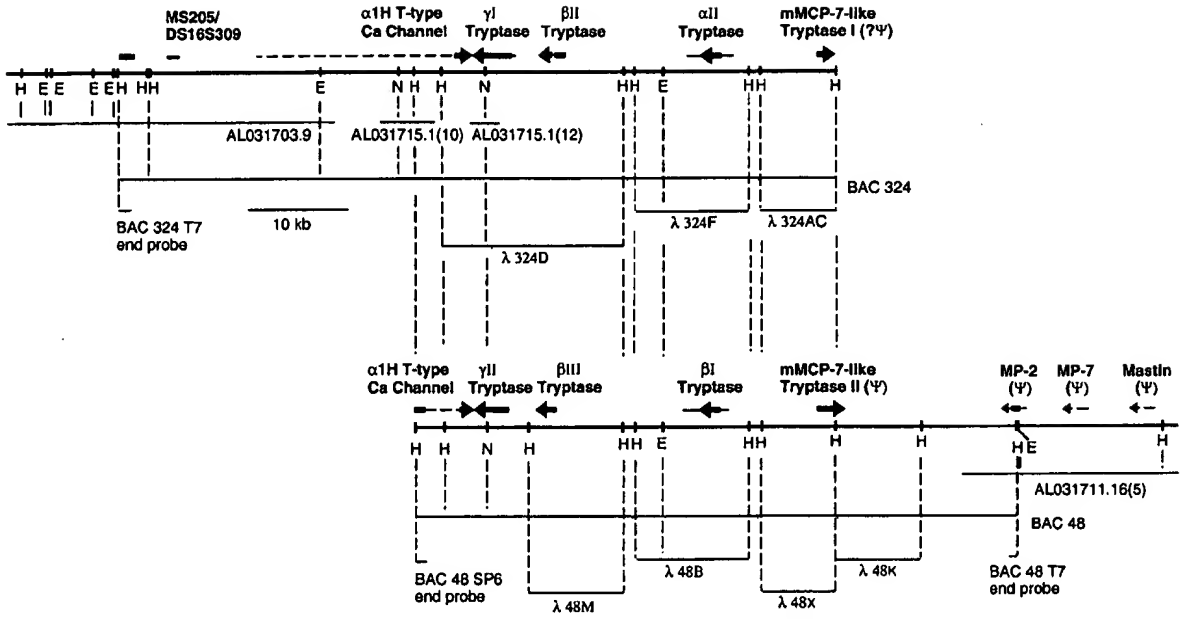

FIGURE 4. Map and alignment of tryptase loci on chromosome 16p13.3. γ-Tryptase genes I and II localize to homologous 16p13.3 loci. These loci are aligned here based on similarities of restriction maps generated using *Hin*dIII (H), *Eco*RI (E), and *Not*I (N), as well as on similarities in sequenced portions of each locus. The map of each locus is primarily based on characterization of BAC 324 and 48, as shown. Portions of each BAC were subcloned as *Hin*dIII restriction fragments, as indicated by the λ clones below each BAC. The sequenced ends of each BAC were used to generate probes, which reinforced the alignment. Each locus was extended by overlap with portions of chromosome 16 sequenced by other groups. The GenBank accession and version numbers of these sequences (e.g., AL031703.9) are given. Where aligned sequence corresponds to fragments of unfinished contigs, the fragment number is indicated in parentheses. Bold lines indicate portions of the BAC that were sequenced. Arrows indicate the predicted direction of gene transcription and the approximate extent of the gene. The thin lines on the sides of the αII and βI genes indicate the extent of sequenced flanking regions. Dotted lines indicate the gene sequence predicted from portions of the genome sequence deposited in GenBank by other investigators. These findings indicate that the γI-tryptase gene is flanked by a βII-tryptase gene and an α1H T-type Ca^{2+} channel gene and is within 30 kb of the highly polymorphic minisatellite, MS205. The γII gene is flanked by a βIII-tryptase gene and a α1H T-type Ca^{2+} channel gene. These loci appear to have arisen from duplications of portions of chromosome 16p.

sequence (exons 2–6) of a gene that is homologous to the γI- and γII-tryptase genes described here. However, two of the exons contain premature stop codons. It remains to be established whether this gene is an allele of γI and γII or is a gene at a separate site; in either case, assuming that this unfinished sequence is accurate, it most likely corresponds to a pseudogene, given the early stop codons. The simplest explanation of our data generated from BACs 324 and 48 and from blotting of full genomic DNA is that there is one genomic γ site with multiple alleles. As shown in Fig. 6 and discussed in the accompanying legend, the organization of γI/γII introns and exons more closely resembles that of the human prostasin gene (46) than of α/β-tryptases, which are more compact genes featuring only five protein-coding exons, with exon 1 consisting entirely of the 5′ untranslated region. Prostasin and the γ-tryptase genes each distributes DNA encoding the prepropeptide over the first three exons, with conserved intron size, phase, and placement. Additionally, prostasin and γ-tryptase genes each encodes a C-terminal, putative membrane-anchoring segment not found in any of the known α/β-tryptases. Even though the nearest neighbors of γ-tryptase genes are α/β-tryptases and they share certain tryptase-like features, it is possible that γ-tryptases are phylogenetically more closely related to prostasin than to α/β-tryptases. It is also possible that ancestors of both prostasin and α/β-tryptases have contributed to the present γ-tryptase genes through recombination, exon swapping, and gene conversion mechanisms, such as those suggested to lead to generation of human mMCP-7-like tryptases, which appear to be chimeras of α/β-tryptase genes and an ancestral mMCP-7-like gene (23).

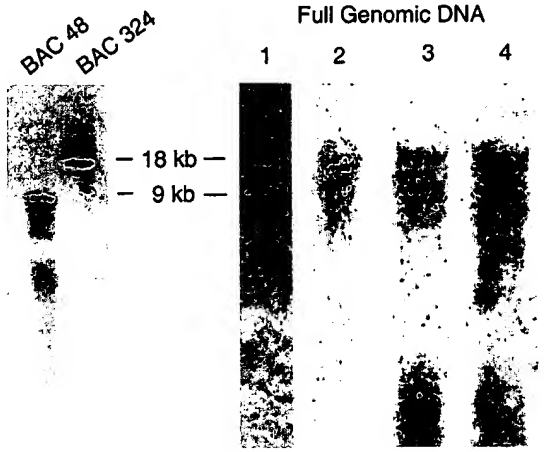

FIGURE 5. Detection of γ-tryptase genes by genomic DNA blotting. BAC 48, BAC 324, and full human genomic DNA were digested with *Hin*dIII, electrophoresed in agarose, blotted, then hybridized with a radiolabeled, 250-bp probe corresponding to a portion of exon 6 on the 3′ side of the *Not*I site (see Fig. 4). As shown by the autoradiogram on the left side of the figure, BACs 48 and 324 yield bands of ~9 kb and 18 kb, respectively, as predicted by the restriction maps in Fig. 4. The right side of the figure shows autoradiograms of similarly blotted and probed genomic DNA from pooled human kidney (Clontech) and peripheral blood leukocytes of three unrelated individuals in *lanes 1–4*, respectively. DNA from pooled kidney and from one individual yields two hybridizing bands (~9 and 18 kb). DNA from two of the individuals yields only the 18-kb band. These data suggest that full genomic DNA contains genes corresponding to both γI- and γII-tryptase in some, but not all, individuals and supports the hypothesis that γI and γII are allelic variants of the same gene.

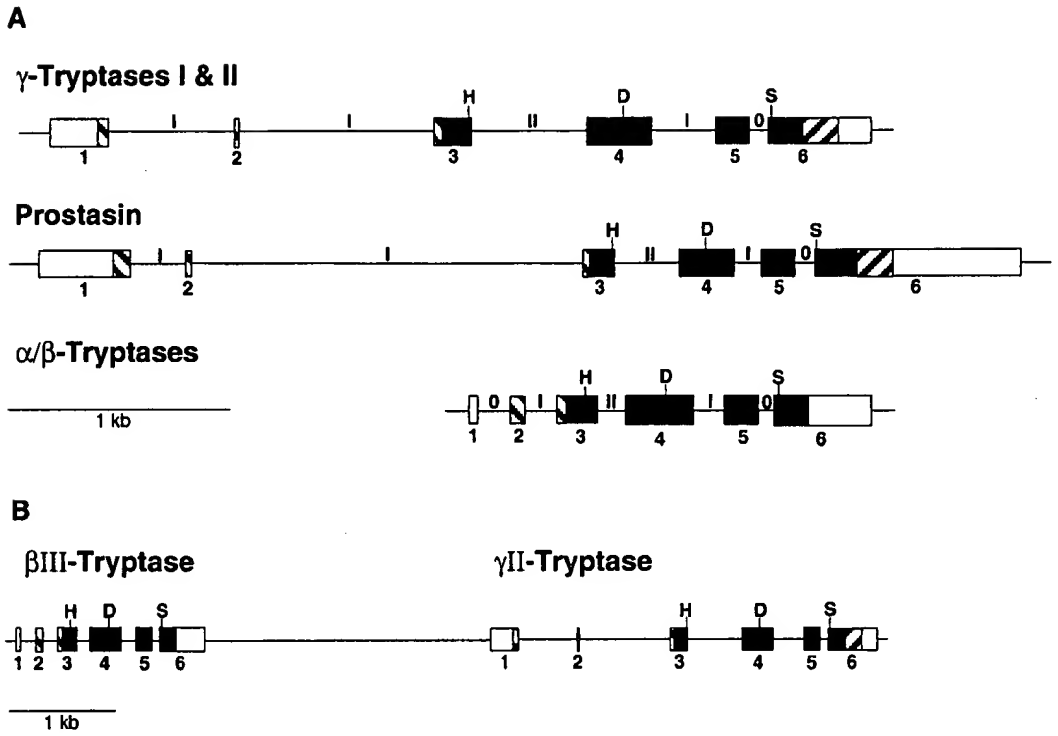

FIGURE 6. Comparison of gene structures. *A*, A comparison of structures of γ-tryptase, prostasin, and α/β-tryptase genes. *B*, A reduced-scale comparison of *A* showing the relationship between BAC 324 βIII and γII genes, which have the same transcriptional orientation and are ~2.5 kb apart. Exons of γ-tryptase, prostasin, and α/β-tryptase genes are numbered and indicated by boxes (□, untranslated; ▨, prepro; ■, catalytic domain; ▨, transmembrane tail). Lines represent introns and flanking regions. The location of codons encoding the His (H), Asp (D), and Ser (S) catalytic triad residues common to serine proteases are indicated. Each intron is labeled with its phase (0, I, or II). Precise lengths of γ-tryptase gene untranslated regions are not yet determined. Note the similarity in phase and placement of introns in γ-tryptase and prostasin genes, both of which contain a prepro sequence divided among three exons. The γ-tryptase and prostasin genes also both contain an extended 3′ open reading frame, which encodes a putative transmembrane segment and small cytoplasmic tail. α/β-Tryptase genes (e.g., βI, βIII, αII) differ in that the 5′ untranslated region is separated from the first protein-coding exon by an intron, the prepro sequence is divided among just two exons, and there is no C-terminal transmembrane tail. These findings suggest a close evolutionary relationship between the γ-tryptase and prostasin genes.

Human mastin gene organization, localization, and amino acid sequence

The sequence of a putative human homologue (not shown) of dog mastin was predicted from the GenBank-derived genomic sequence. Intron splice junctions were predicted by reference to dog mastin cDNA (27) and patterns of intron phase and placement in known tryptase genes (22, 23) by examination of open reading frames and by application of "GT. . . AG" rules for intron ends, as noted above in connection with γ-tryptase genes. As shown in Fig. 4, genomic sequences more homologous to dog mastin than to any other known cDNA were identified in fragment 5 of partially sequenced contig AL031711.16, which overlaps extensively with BAC 48's T7 end. However, no closely related human ESTs were found. The gene itself is flawed by faulty splice junctions, by mutation of Ser^{195} to Asn (which would lead to catalytically inactive protein if the gene were expressed), and intronic insertions of Alu repetitive sequences. Thus, this mastin-homologous gene is a pseudogene, which may explain the absence of human ESTs and the failure of polyclonal antisera raised against dog mastin (28) to identify homologues in human tissues and cell lines (Ref. 29 and our unpublished results). Two additional apparent tryptase pseudogenes (MP-2 and MP-7; see Fig. 4) lie in the same fragment of AL031711.16. BAC 48's T7 end terminates at a *Hin*dIII site in a minisatellite in the middle of MP-2 pseudogene intronic sequence. Thus, both BACs and their flanks are rich in tryptase-like genes and pseudogenes. Based on the sequence acquired so far, these regions of chromosome 16 are also richly endowed with various repeats, at least one of which, MS205, is highly polymorphic. DNA instability in the neighborhood of these repeats may contribute to general instability in the region, facilitating duplications, gene conversion events (23), and proliferation of pseudogenes.

γ-Tryptase protein expression

Polyclonal Abs raised against a portion of the γ-tryptase catalytic domain sequence recognize a protein of ~31 kDa in extracts of HMC-1 5C6 cells, as shown in Fig. 7. This band is distinct from those of β-tryptases based on its smaller apparent size, narrower banding pattern, and lack of reactivity with antisera raised against purified human lung mast cell tryptase (not shown). These results suggest that one or more γ-tryptases are expressed in 5C6 cells and predict that mast cells are a source of this enzyme. The detection of γ-tryptase mRNA in a variety of tissues by PCR, but not by conventional mRNA blotting, is consistent with the expression of γ-tryptase in a subpopulation of cells found in many tissues, such as mast cells. No difference in migration of the immunoreactive band was found in the presence or absence of a reducing agent, suggesting the absence of disulfide-linked oligomerization characteristic of some tryptase-like enzymes, such as mastin (29). The lack of a reduction in size in the presence of reducing agents suggests that the predicted disulfide-linked propeptide remnant is absent in the mature enzyme or that it is too small to produce detectable differences in electrophoretic migration between reduced and unreduced samples.

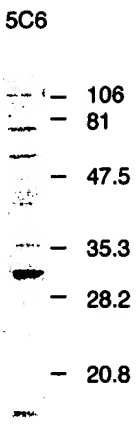

FIGURE 7. Immunodetection of γ-tryptase. Proteins detergent-extracted from HMC-1 5C6 cells were separated by SDS-PAGE, blotted, and hybridized with chicken antiserum raised against a synthetic peptide corresponding to a predicted γ-tryptase surface loop. Bands recognized by the polyclonal Abs were visualized using goat anti-chicken IgG secondary Abs conjugated to alkaline phosphatase. As seen in the developed blot, there is a prominent band of ~31 kDa along with various minor bands that may represent nonspecific interactions. The ~31-kDa band is absent in blots incubated with preimmune serum (not shown). The elution position and size (in kDa) of marker proteins is indicated to the right of the blot.

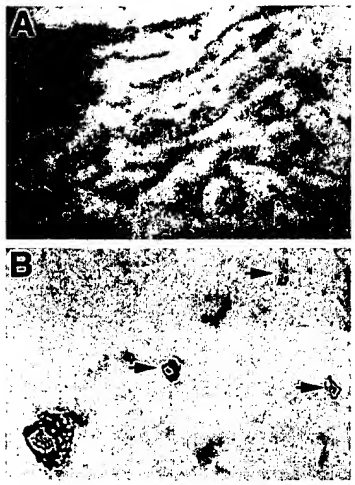

FIGURE 8. Human airway expression of γ-tryptase. Cryosections of human trachea were screened for cells immunoreactive for γ-tryptase using the same polyclonal anti-γ-tryptase Ig used to screen the protein blots in Fig. 7. *A*, A section of human airway submucosa incubated with nonimmune serum (original magnification, ×20). No strong immunoreactive cells are observed. *B*, Submucosa from the same block of human airway incubated with anti-γ-tryptase IgY (original magnification, ×40). Scattered mononuclear cells (arrows) with granular immunoreactive inclusions are seen. The inset in the lower left corner is an enlarged view (original magnification, ×100) of the central cell in the panel. In both sections, the secondary Ab was alkaline phosphatase-conjugated goat anti-chicken IgY; alkaline phosphatase activity was detected using 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium.

As shown in Fig. 8, our polyclonal anti-γ-tryptase Abs recognize scattered, granulated mononuclear cells in human airway submucosa. The distribution and appearance of these cells is typical of tissue mast cells, which is consistent with the identification of immunoreactive γ-tryptase in the 5C6 subclone of the HMC-1 line of mast cells. The granular pattern of staining suggests the possibility that immunoreactive γ-tryptase resides in secretory granules.

γ-Tryptase homology model

Models of the γI-tryptase catalytic domain based on the established structure of its closest crystallized relative, βII-tryptase, are depicted in Fig. 9. These models reveal that the segment Cys^{192}-Pro^{206}, which is among the most distinctive compared with otherwise similar relatives (see Fig. 1), forms a predicted surface loop that is far enough removed from the classic serine protease substrate binding site that it is unlikely to interact directly with polypeptide targets in the vicinity of the scissile bond. Because this is the peptide segment against which antisera were raised, it is unlikely that our anti-γI-tryptase Ig will block access to potential peptide substrates. The models also show that the sole predicted N-glycosylation site is closer to the catalytic active site. However, this glycosylation site, which is not conserved in α/β-tryptases, prostasin, or testisin, lies outside of the classic serine protease-extended peptide binding site. Carbohydrate attached at this site could interfere with bulky protein substrates but probably not with smaller peptide substrates. The models also predict that the two cysteines (145 and 154) that we are unable to pair with other cysteines based on the βII-tryptase crystal structure are not close enough to form a disulfide linkage with each other. Both lie on predicted surface loops and could potentially form intersubunit bonds with other catalytic subunits. However, there is no evidence of this in HMC-1 immunoblots. As noted above, we predict that Cys^{145} (Cys^{122} in chymotrypsinogen) may remain linked to cleaved remnants of propeptide by analogy to chymotrypsin. The model also predicts that the putative membrane-anchored C-terminal hydrophobic segment attaches to the catalytic domain's back side, where it is less likely to interfere with substrate access to the active site.

Relation of γ-tryptases to transmembrane tryptase

While this manuscript was under review, Stevens and colleagues published a report of a similar gene, termed transmembrane tryptase (47). Their gene contains one fewer exon and is ~0.8 kb shorter than our γ genes. However, the deduced amino acid sequence of transmembrane and γ-tryptase catalytic domains is 98–99% identical, suggesting that the enzymes may be allelic variants of each other. Partial characterization by Stevens and colleagues of a gene locus containing a mouse homologue closely related to that predicted from our murine ESTs suggests that the mouse gene has a relationship to the neighboring mouse tryptase mMCP-6 gene that is similar to the relationship between the human γII and βIII tryptase genes we describe here. Interestingly, Abs raised against transmembrane tryptase recognize mast cells in human skin and intestine, thereby supporting our hypothesis that γ-tryptase is expressed in mast cells based on our identification of immunoreactive protein in human airway cells and in a mast cell line. If, like other human tryptases, γ-tryptases are expressed in most mast cells, then γ-tryptase expression in tissues will be widespread. The immunological significance of γ-tryptase expression by mast cells is not yet clear and awaits further characterization of this novel gene product's biogenesis and physical and enzymological properties. If γ-tryptases, like mast cell β-tryptases, are activated intracellularly, stored in secretory granules, and secreted in response to Ag-bound IgE, they may aggravate the local pathology of allergic inflammation by hydrolyzing proteins in the vicinity of degranulated mast cells. In this regard, if γ-tryptases remain membrane-anchored after activation and release, they may remain attached to the cell surface, thus ensuring that proteolytic effects

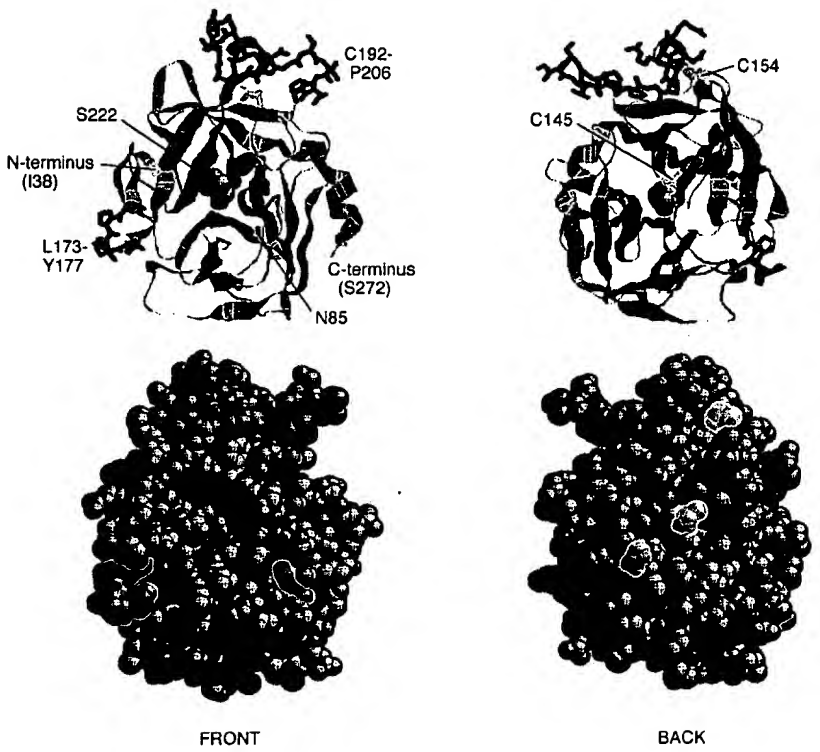

FIGURE 9. Molecular models of γ-tryptase. A homology model of human γI catalytic domain was constructed using Swiss-Model. Coordinates of human βII tryptase (1AOL) (34) served as template for the model. Images were generated using RasMac (version 2.6-ucb.1, available at http://mc2.cchem.berkley.edu/rasmol/v2.6/). The top pair of ribbon models begin with Ile^{38} and end in Ser^{272} at the C terminus. The N and C termini are colored orange. The active site faces front in the left panel views, with the catalytic serine colored red in space-filling mode. The right panels show the back of the protease rotated 180° on a vertical axis compared with the front views. For emphasis, side chains are shown for Leu^{173}-Tyr^{177} (involved in oligomerization in α/β-tryptases; colored green), Cys^{192}-Pro^{106} (the peptide against which Abs were raised; colored blue), Asn^{85} (a consensus N-glycosylation site; cyan), and cysteines 145 and 154 (neither of which is found in α/β-tryptases; yellow). The same color scheme applies to the space-filling models in the lower half of the figure. The predicted γ-tryptase C-terminal hydrophobic tail is not present in βII-tryptase and therefore is not modeled here. Note that it is expected to extend from the back of the protease, allowing the active site to face away from the membrane. In the front view, the putative substrate binding site extends roughly vertically above and below the active site Ser^{222}.

remain local. However, if γ-tryptases are released from their membrane anchors, secretion may allow the enzymes to reach targets that are remote from the site of mast cell stimulation.

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targeting vector do not express neomycin resistance. The 999,999 failures are killed by the antibiotic. Only the ES cells that express the neomycin resistance gene product will successfully resist the antibiotic in the tissue culture medium, survive, and grow into colonies. Visible colonies of ES cells growing after the neomycin treatment are harvested as candidates likely to contain the mutated gene in their genome.

Positive ES cells are implanted into **blastulas** from normal mice. Blastulas are collected from superovulated female mice, often of the C57BL/6 inbred strain. Collection is usually performed at embryonic day 3, when the blastula is at a very early stage of development. A fine-gauge needle is used to microinject ES cells from the tissue culture colony into the central hollow blastocoele of each blastula. The injected blastulas are then implanted into **pseudopregnant female recipients.** These adoptive mothers can be of any strain. C57BL/6J and CD-1 are often used as recipients, as they sustain pregnancies well and provide good parental care. The stages of the knockout technology are diagrammed in Figure 2.3.

THE CHIMERA

Successful microinjection of ES cells into the blastocoele results in an embryo comprised of cells from the original blastula plus the ES cells implanted into the blastula. Cells of both origins grow in concert to form one complete embryo. The resulting mouse pup has tissues and organs composed of a mosaic mixture of cells derived from the original 129 ES cells and the original C57BL/6 blastula cells. The pup is called a **chimera** because it contains cells from two independent sources.* The C57BL/6 strain has black fur. The 129 strain has light grayish-brown fur. The coat color of chimeric pups is grayish brown, sometimes a mosaic of black and grayish-brown patches or stripes. If no ES cells were incorporated at the blastula stage, the pup will show a black coat color. The appearance of the fur is a useful early marker of a successful mutation. Chimeras are identified as soon as coat color is detectable, at about 3 weeks after birth.

F_1 AND F_2 OFFSPRING

The targeted gene construct is incorporated into the blastula at random locations. When incorporation is only in the somatic cells that develop into nonreproductive tissues, the original chimeras express the mutation, but their offspring do not. When incorporation is in cells of the blastula that develops into germ-line gametes, that is, eggs and sperm, the targeted mutation is transmitted to the next generation of the offspring of the chimera. The scheme for generating chimeras and their offspring is shown in Figure 2.4.

To detect germ-line transmission, a test cross is conducted. The chimera is bred to a mouse of a normal inbred strain, such as C57BL/6J. The F_1 offspring of the test cross are analyzed for expression of the mutation. An F_1 offspring that receives the mutated gene from the chimeric progenitor parent is heterozygous for the mutated gene. Southern blot analysis or polymerase chain reaction assay is performed on a small tissue sample from the

*In Greek mythology, the Chimera was a fire-breathing monster: a lion in the front, a goat in the middle, and a serpent in the back. Bellerophon killed the Chimera by thrusting lead down its throat. Its fiery breath melted the lead, which trickled down into the stomach and killed the Chimera, saving the Kingdom of Lycia (D'Aulaire and D'Aulaire, 1962).

CRAWLEY, "WHAT'S WRONG WITH MY MOUSE" (WILEY-LISS 2000)

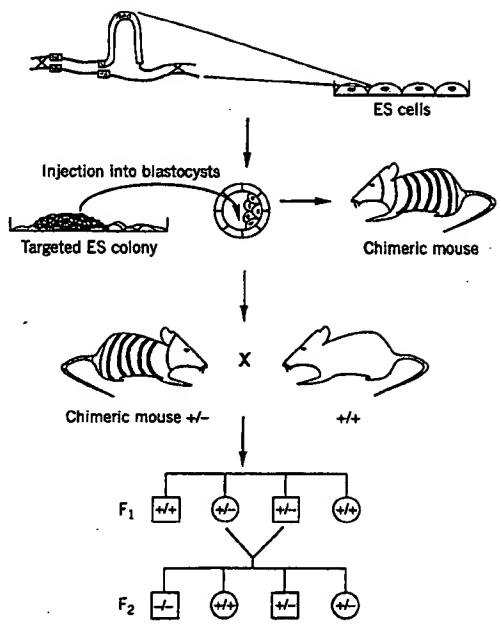

Figure 2.4 *Chimeric mice grow from the blastulas containing embryonic stem cells expressing the targeted gene construct. When the mutation is expressed in germ-line cells of the chimera, the mutation is transmitted to subsequent generations.* [From Wehner and Silva (1997), p. 244.]

tail of the offspring, to identify positive heterozygotes. Each positive heterozygote is a potential founder for a line of mutant mice.

Identified F_1 heterozygote offspring are mated with each other to produce an F_2 generation. Theoretically, the F_2 population will follow the principles of Mendelian segregation, resulting in one-fourth (1/4) homozygous mutants (−/−), one-half (2/4) heterozygotes (+/−), and one-fourth (1/4) homozygous wildtype controls (+/+). If the gene is lethal, the homozygotes will not survive. If the gene is located on the X or the Y chromosome, gender issues influence the ratio of males and females for each genotype.

The genotypes of the F_2 mice are tested by Southern blot for the presence of the normal gene in the +/+ mice, the presence of half the normal complement of the gene in the +/− mice, and the absence of the gene in the −/− mice. The expression of the gene product, when the gene product is known, is assayed by an appropriate technique, such as high-pressure liquid chromatography for an enzyme. The heterozygotes (+/−) may express half the gene product, reflecting the presence of half the gene dose, or may express variable amounts of the gene product in some cases. The homozygous mutant mice (−/−) should show no gene product. These confirmed −/− mice are termed the **null mutants.** Techniques for confirmation of the mutation are illustrated in Figure 2.5.

Field: **Author,** Limits: **Publication Date from 1994 to 1994**

1: J Immunol. 1994 Feb 15;152(4):1907-14.

Mast cell phagocytosis of FimH-expressing enterobacteria.

Malaviya R, Ross EA, MacGregor JI, Ikeda T, Little JR, Jakschik BA, Abraham SN.

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Most studies of mast cells have been directed at their role in the pathophysiology of IgE-mediated allergic reactions with little recognition of their participation in bacterial infections. We report that mast cells can specifically bind FimH, a mannose-binding subunit on type 1 fimbriae expressed by Escherichia coli and other enterobacteria. This interaction triggers mast cell phagocytosis and killing of the bacteria within vacuoles and through the release of superoxide anions. Also, in view of the fact that mast cells have the capacity to release inflammatory mediators and are particularly abundant in the skin, mucosal surfaces, and around blood vessels, we suggest that these cells play an important role in host defense against microbial infection.

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